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Using Head Patch Pattern as a Reliable Biometric Character for Noninvasive Individual Recognition of an Endangered Pitviper *Protobothrops mangshanensis*

Daode YANG[1*], Sikan CHEN[1], Yuanhui CHEN[2] and Yuying YAN[1]

[1] *Institute of Wildlife Conservation, Central South University of Forestry and Technology*, *Changsha* 410004, *Hunan*, *China*
[2] *Hunan Mangshan National Nature Reserve Administration*, *Yizhang* 424221, *Hunan*, *China*

Abstract Mangshan pitviper, *Protobothrops mangshanensis* (formerly *Zhaoermia mangshanensis*) is endemic to China. Unfortunately, due to the decreasing size of its wild populations, this snake has been listed as critically endangered. Research carried out on the Mangshan pitviper's population ecology and captive reproduction has revealed that the unique head patch patterns of different individuals may potentially be used as a noninvasive recognition biometric character. We collected head patch pattern images of 40 individuals of *P. mangshanensis* between 1994 and 2011. By comparing each pitviper's head patch pattern, we found that the head patch pattern of individual snakes was different and unique. Additionally, we observed and recorded the head patch pattern characters of four adults and five juveniles before and after ecdysis. Our findings confirmed that head patch patterns of Mangshan pitvipers are unique and stable, remaining unchanged after ecdysis. Thus, individuals can be quickly identified by examining the head patch pattern within a specific recognition area on the head. This method may be useful for noninvasive individual recognition in many other species that display color patch pattern variations, especially in studies of endangered species where the use of invasive marking techniques is undesirable.

Keywords biometric identification, endangered snake, head patch pattern, Mangshan pitviper, noninvasive individual recognition, image analysis, natural markings

1. Introduction

Biometric recognition using common biological characteristics can be applied to identify individuals of species that exhibit stable differences in those characteristics (Jain *et al*., 2004). Accurately marking patterns and identifying individual animals are vitally important in wildlife management and conservation research. Managers and researchers can save time and energy, and improve the accuracy of estimates based on individual identification, by checking natural marks to identify individuals (Pollard *et al*., 2010). Biometric recognition techniques have been used successfully in studies of larger mammals (Katona and Whitehead, 1981; Kelly *et al*., 1998; Neuhaus and Ruckstuhl, 2002), and also in studies of some smaller vertebrates (Bradfield, 2004; Gamble *et al*., 2008; Costa *et al*., 2009; Angelini, 2010; Hoque *et al*., 2011). But identification of individual snakes is problematic (Shine *et al*., 1988), especially in rare and venomous species (Pendlebury, 1972; Moon *et al*., 2004). The recognition methods used to identify individual snakes in previous studies have relied heavily on artificial marking techniques such as scale-clipping (Blanchard and Finster, 1933; Brown and Parker, 1976; Spellerberg, 1977), painting (Hirth, 1966), tagging (Pough, 1970; Pendlebury, 1972; Rodda, 1988), heat-branding (Clark, 1971), freeze-branding (Lewke and Stroud, 1974), and using implantable microchips (Elbin and Burger, 1994). Some of these methods are useful, but many are limited by detrimental factors such as the

* Corresponding author: Prof. Daode YANG, from Central South University of Forestry and Technology, Changsha, Hunan, China, with his research focusing on wildlife ecology and nature reserve management.
E-mail: csfuyydd@126.com
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extra handling involved, the requirements for expensive professional tools, and the disappearance of marks after ecdysis (Blanchard and Finster, 1933).

The Mangshan pitviper, *Protobothrops mangshanensis* (formerly *Zhaoermia mangshanensis*; Gumprecht and Tillack, 2004) is only found in the Nanling Mountains of southern Hunan, China (Zhao and Chen, 1990; Guo *et al*., 2007; Orlov *et al*., 2009). This critically endangered species is the largest of the Viperidae in China, and is of high scientific value, especially with regards to conservation management (Gong *et al*., 2013). Our study on the population ecology and captive reproductive biology of *P. mangshanensis* has revealed that the individuals of this snake have a unique head patch pattern that can be used as an accurate and noninvasive recognition biometric character. Additionally, although there have been several studies on recognition techniques for identifying individual snakes (Pendlebury, 1972; Moon *et al*., 2004), the precision achieved by using biometric characters has rarely been reported. Here, we describe the biometric recognition area on the head of *P. mangshanensis* and demonstrate the effectiveness of using this technique for accurate long-term identification of individual snakes. This method could be used for long-term individual identification of many other reptile and amphibian species with typical patch patterns that exhibit stable individual differences.

2. Material and Methods

2.1 Collecting head pattern images We caught wild Mangshan pitvipers from the Mangshan National Nature Reserve in the Nanling Mountains, Hunan, China using professional capture tools such as hooks and pincers. After photographing each pitviper's head patch pattern (see examples in Figure 1), all were released back to their capture sites except for five adult females. These females were maintained in captivity to observe whether their head patch patterns remained stable over time.

In total, we collected head patch pattern images from 31 wild-caught adult individuals of *P. mangshanensis* between 1994 and 2011. Additionally, we were able to photograph head patch patterns of nine neonates (1 male, 8 females) that hatched in 2011, (produced by one of the captive female pitviper). From analyzing close-up photos of the dorsal head surface, we were able to define a concise "recognition area" (Figure 2).

2.2 Pre-processing and matching head patch pattern images In order to determine whether individual snakes could be correctly identified on the basis of head patch patterns, we divided the snake head images into a multiple square lattice and numbered every square in a 10 × 10

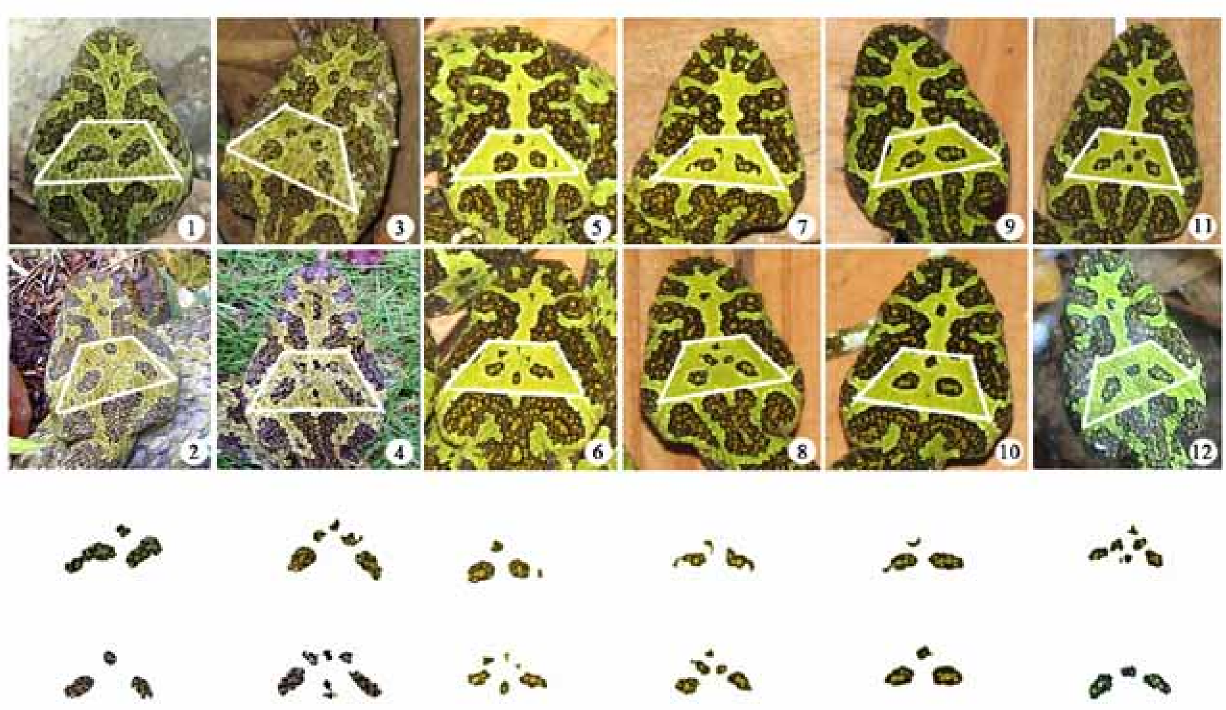

Figure 1 Variety of differences in the unique head patch patterns found in the recognition area of individual *Protobothrops mangshanensis* (n = 12). Images 1–4 show four adult females; images 5–12 show juveniles (5 females, 1 male).

Figure 2 The trapezoidal "recognition area" on the head of *Protobothrops mangshanensis* showing the unique head patch pattern.

array (Figure 3). We measured each square side length (l) and the length from snout to neck (L). Thus, every square lattice side length could be calculated as $l = 1/10L$. This method was used to avoid errors resulting from the differences in the relative position of head patch patterns of snakes with different head sizes.

The numbered 10 × 10 array provided a unique "serial number" of head patch patterns for each snake. For example, the snake in Figure 3 has the following head patch "serial number" (-3, 4; -2, 4; -2, 5; -1, 4; -1, 5; 1, 4; 1, 5; 1, 6; 2, 4; 2, 5).

2.3 Pattern recognition before and after ecdysis To determine if head patch patterns were stable over time, we closely monitored the head patch pattern characteristics of four adult females and five juveniles (4 females, 1 male) before and after ecdysis. The first adult female molted seven times between October 2007 and June 2010, the second adult female five times between July 2010 and November 2011, the third adult female three times between March 2011 and November 2011, and the fourth adult female once between August 2011 and November 2011. Of the five juveniles, two females molted twice, and the other three (2 females, 1 male) molted once each between September 2011 and April 2012.

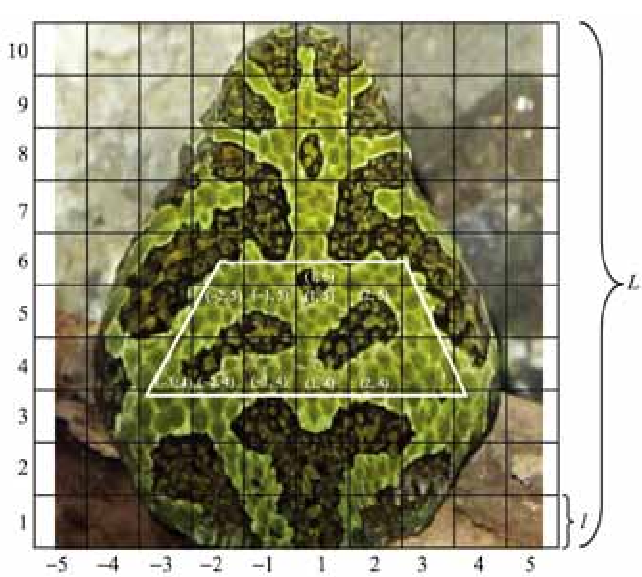

Figure 3 Pre-processing the head images showing how to analyze differences in head patch patterns using the unique "serial number" derived from the numbered square lattice.

3. Results

3.1 Recognition area and head pattern characteristics The biometric recognition area of Mangshan pitvipers is located on the top of the head between the two venom glands, as indicated by the light-colored "trapezoid" in Figure 2. The recognition area in all observed individuals (n = 40) contained 2–7 irregular dark green patches, with most individuals exhibiting 3–4 dark patches (Figure 4). Thus, we set up six databases named according to the number of head patch patterns of Mangshan pitvipers. For example, information from pitvipers with four head patches was entered in the No. 4 database, those with 5 head patches into the No. 5 database and so on.

3.2 Stability of head patterns Our careful monitoring of captive individuals showed that head patch patterns within the recognition area were not affected by ecdysis or the number of molting events over time. Head patch patterns maintained a high level of consistency and stability before and after ecdysis (Figure 5).

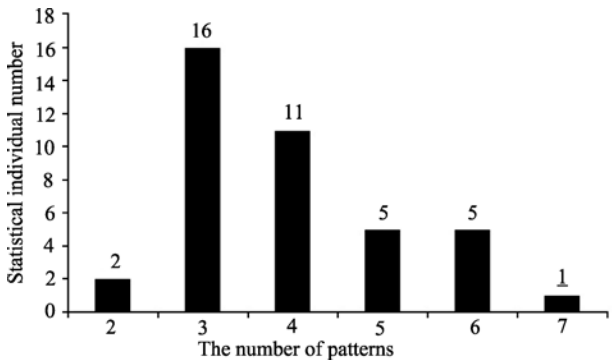

Figure 4 Histogram showing the number of dark patches in the recognition area of individual *Protobothrops mangshanensis* (n = 40).

3.3 Patch pattern image analysis By comparing the "serial numbers" of head patch patterns for every snake in each database, we found that the head patch pattern information of every individual was unique. The number, shape, size and relative positions of head patch pattern characters within the recognition area allowed us to quickly and accurately identify each of the 31 adults and nine juveniles of Mangshan pitvipers for which we recorded head pattern data (Figure 6).

4. Discussion

Most methods used to mark snakes are invasive to some extent. Techniques such as scale-clipping, tagging, heat-branding, freeze-branding and using implantable microchips require professional skills and equipment, and can be timely and expensive (Elbin and Burger, 1994). Additionally, using these techniques for animals which need to be caught and held can be stressful for the animals, and risky for people, especially when venomous snakes need to be handled (Pendlebury, 1972; Moon *et al*., 2004). Less invasive methods such as paint-marking may be useful for short-term studies (Hirth, 1966), but cannot be used to identify individuals permanently because the markings disappear due to ecdysis (Blanchard and Finster, 1933). Thus, the head patch pattern recognition method we describe here may be the easiest way to recognize individual snakes permanently, in a noninvasive manner. It does not harm the snakes or affect their behavior, and capturing the snakes is not required.

Recent advances in the development of biometric technology have seen a rapid rise in the implementation of fingerprint, face and iris recognition for use in various fields, including personal identification, verification, and security (Jain *et al*., 2004). The metho -dology involves collecting a database of images so that features can be analyzed and compared to achieve identification (Ojala *et al*., 1996; Permuter *et al*., 2006). The head patch pattern recognition method we tested with *P. mangshanensis* is conducive to establishing

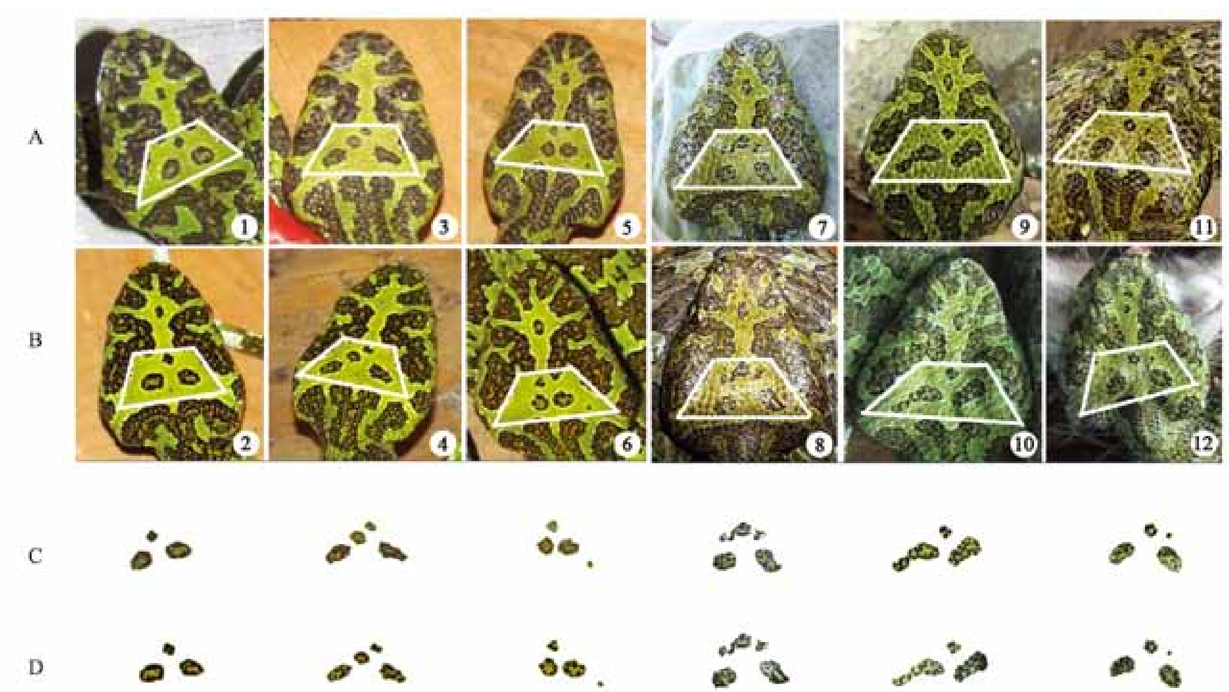

Figure 5 Stability of the head patch patterns of *Protobothrops mangshanensis* before (A) and after (B) ecdysis (n = 6). Images 1–6 show juveniles of about 5 months old (5 females, 1 male); images 7–12 show adult females of about 5–6 years old.

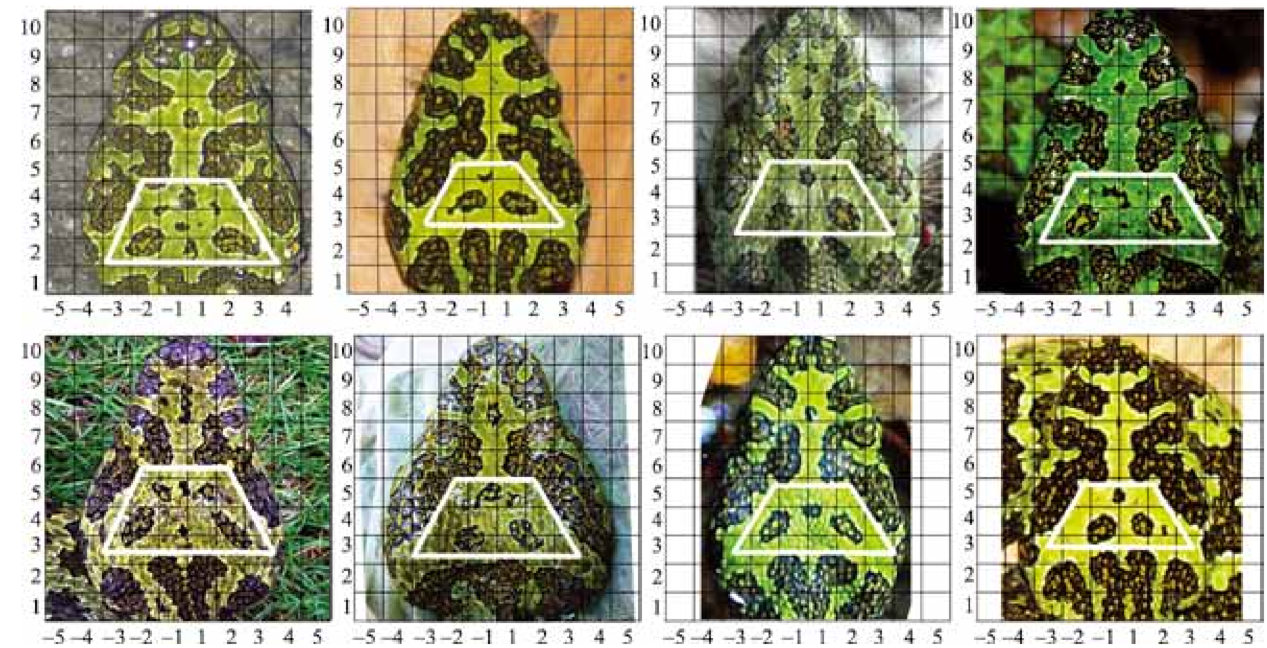

Figure 6 Head patch pattern images of Mangshan pitvipers after the pre-processing stage showing the unique square lattice serial numbers of each snake.

a head patch pattern database. Thus, with computer analysis of such a database it may be possible to use biometric methods in combination with other biological characteristics, such as body size, sex, and age, to further increase the speed and accuracy of individual snake identification. A recent study tested the feasibility of automated biometric identification of newts by matching their unique dorsal patterns using computer vision techniques. The results are promising for the future use of such technology in rare and endangered species identification (Gamble *et al*., 2008).

Biometric recognition methods have successfully been used for the individual identification of large animals, such as cheetah *Acinonyx jubatus* (Kelly *et al*., 1998), zebra *Equus zebra* (Neuhaus and Ruckstuhl, 2002), and humpback whales *Megaptera novaeangliae* (Katona and Whitehead, 1981). Additionally, a recent study showed that such identification methods might also be useful for study on smaller animals. For example, the color pattern of individuals of rock shrimp (*Rhynchocinetes typus*) was found to persist after one molt and all 14 individuals in the study were able to be positively identified based on their color patterns (Gallardo-Escárate *et al*., 2007).

To date, however, there have only been a few studies on the use of natural patterns as an aid to identifying reptiles (Moon *et al*., 2004) or amphibians (Bradfield, 2004; Hoque *et al*., 2011). In China, newts and salamanders, such as *Cynops, Paramesotriton*, *Pachytriton* and *Hypselotriton*, exhibit irregular patterns on the abdomen (Fei *et al*., 2010). Similarly, Chinese toads (*Bufo raddei*) and frogs (*Kaloula pulchra*, *Microhyla pulchra* and *Odorrana lungshengensis*), exhibit irregular patterns on their dorsal surfaces (Fei *et al*., 2010). Many other Chinese reptiles also exhibit individual patterns. The Fea viper, *Azemiops feae* has irregular patterns on the head, and the Chinese crocodile lizard, *Shinisaurus crocodilurus* exhibits irregular patterns on the rump (Wang, 2011). Our finding that the unique head patch patterns of *P. mangshanensis* provide a permanent noninvasive identification biometric character for identification of adult and juvenile snakes suggests this method could be used for long-term identification of many reptile and amphibian species worldwide.

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